

# Food Risk Analysis Communication

Issued By Health Canada's Food Directorate

---

## Health Canada's Proposal to Accept a Health Claim about Soy Products and Cholesterol Lowering

Karima Benkhedda<sup>1</sup>, Cynthia Boudrault<sup>1</sup>, Susan E. Sinclair<sup>1</sup>, Robin J. Marles<sup>1</sup>, Chao Wu Xiao<sup>1</sup> and Lynne Underhill<sup>1</sup>

<sup>1</sup> Bureau of Nutritional Sciences, Food Directorate, Health Products and Food Branch, Health Canada

Health Canada's Food Directorate is making available this paper, following an internal peer review by Food Directorate scientific and regulatory experts, to seek comments from peer scientists, regulators and stakeholders prior to finalization.

This paper is open for comment commencing October 22, 2014, and closing on November 21, 2014 (30 calendar days). Comments of a scientific nature only will be considered in developing the final version of this document. Authors will strive to document how the various comments received, when deemed relevant, were considered in amending and shaping the final published document.

Comments may be submitted electronically at the address indicated below. Please use the phrase "Soy Health Claim Comments" in the subject box of your e-mail.

E-mail: [healthclaims-allegationssante@hc-sc.gc.ca](mailto:healthclaims-allegationssante@hc-sc.gc.ca)

DOI: 10.5772/59411

© 2014 Health Canada. Licensee InTech. This is an open access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

---

### 1. Introduction

In Canada, a health claim for a food is any representation in labelling or advertising that states, suggests or implies that a relationship exists between the consumption of a food and health. A therapeutic health claim is a statement that links a food to the treatment or mitigation of a disease or health-related condition, or to restoring, correcting or modifying body functions. A disease risk reduction claim is a statement that links a food to a reduced risk of developing a diet-related disease or condition.

Since 2003, Health Canada's Food Directorate has permitted the use of several disease risk reduction claims through regulatory amendments (B.01.603 of the *Food and Drug Regulations*). In 2010, Health Canada reconsidered the

classification of food products with disease risk reduction claims or therapeutic claims in light of clarified principles for the classification of foods at the Food-Natural Health Product interface. Health Canada's position is that when food products are marketed for a disease risk reduction or therapeutic benefit, which comes as a result of the food's normal use as part of the diet, these products may be classified and regulated as foods. In other words, the use of a disease risk reduction claim or a therapeutic claim alone does not systematically lead to the classification of the product as a drug. As a result, from 2010 to present, Health Canada's Food Directorate has published favourable decisions on disease risk reduction and therapeutic claims without the need for regulatory amendments. Accepted therapeutic claims include cholesterol lowering for plant sterols, oat products, psyllium products, replacement of

saturated fat by mono- and polyunsaturated fat, barley products and ground whole flaxseed [1]. A disease risk reduction claim about a reduced risk of dental caries has also been accepted for sugar-free chewing gum [1].

A request for an opinion on the validity of a therapeutic health claim about protein-rich soy foods and cholesterol lowering was received in December 2011 by Health Canada's Food Directorate. An assessment of this therapeutic claim was conducted to determine its acceptability for foods. The information provided by the petitioner along with other relevant scientific literature was assessed to reach the conclusion outlined in this document.

## 2. Assessment of the Claim Validity

The submission was evaluated against the standards of evidence set out in Health Canada's *Guidance Document for Preparing a Submission for Food Health Claims* [2].

The foods that are the subject of the proposed health claim are foods or food ingredients that contain proteins derived from the soybean (*Glycine max* (L.) Merr., Fabaceae) along with inherent soy isoflavones. In addition to protein, soy contains varying amounts of fat, carbohydrates (including dietary fibre), phytochemicals (such as isoflavones and saponins), and many minerals and vitamins [3]. Some of the foods that could be eligible are whole soy foods such as soy beverages, tofu, miso, tempeh, natto, soy cheese and soy nuts. Others are foods containing processed soy ingredients, including isolated soy protein (ISP), soy protein concentrate (SPC), textured soy protein (TSP) and soy flour (SF). Soy sauce and soybean oil are excluded from the claim because they lack substantial amounts of soy protein.

Epidemiological studies suggest that soy food consumption may be associated with a lower incidence of certain chronic diseases including coronary heart disease (CHD) and with a decrease of some of its risk factors [4, 5]. Elevated blood cholesterol is recognized as an important modifiable risk factor for cardiovascular disease (CVD) [6], which is the second leading cause of death in Canada [7]. Low-density lipoprotein (LDL) is recognized as the major atherogenic lipoprotein fraction [8] and LDL-cholesterol remains the primary target for the risk of CVD in the 2012 Update of the Canadian Cardiovascular Society Guidelines for the Diagnosis and Treatment of Dyslipidemia for the Prevention of Cardiovascular Disease in the Adult [9]. In addition, cholesterol lowering is relevant to the general Canadian adult population on the basis that 39% of Canadians aged 6 to 79 years old had unhealthy levels of total cholesterol (>5.2 mmol/L for adults<sup>1</sup>) during the time period of 2009-2011 [10].

---

<sup>1</sup> To convert total, HDL and LDL cholesterol values in mmol/L to mg/dL multiply by 38.67. To convert triglyceride values in mmol/L to mg/dL, multiply by 88.57.

## 2.1 Literature Search and Results

The petitioner provided a literature search covering the period from 1980 to March 2010 to substantiate the proposed health claim. The literature search was updated by the Food Directorate to March 2013 with no limit to the search period. Medline, Embase, CAB Abstracts, Agricola, Econlit, Food Science and Technology Abstracts, Global Health and International Pharmaceutical Abstracts were searched for English and French articles about soy and cholesterol. References were included if they reported on intervention or observational studies (cohort studies and nested case-control studies) published in a peer-reviewed journal; included a suitable non-soy protein control treatment; quantified soy protein intake; included generally healthy or mildly hypercholesterolemic adult ( $\geq 18$  years of age) men and women not taking any medications known to alter lipid levels; included participants not dieting and not involved in a weight loss program; lasted a minimum of 3 weeks; and reported changes in serum lipids (triglycerides, total, LDL, and/or HDL cholesterol).

A total of 49 relevant references [11-59] were identified, for a total of 79 studies since many references included more than one comparison between a soy food and a control. All references reported on clinical trials. No observational studies met the selection criteria. The outcomes considered were changes in total cholesterol, LDL cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides (TG).

## 2.2 Characteristics of Included Studies

The studies were conducted in normo- and hypercholesterolemic males and females ranging from 18 to 80 years of age. Studies were carried out in the Americas, Europe, Asia and Oceania, with the vast majority conducted in the United States. Twenty studies were conducted in men including 8 in young men, 28 studies were conducted in women including 24 in postmenopausal women, and 31 studies were conducted in both men and women. Treatment duration ranged from 1 to 12 months and the daily intake of soy protein ranged from 11.3 g to 154 g. The majority of studies used doses in the range of 25-50 g/day. The smallest study included 9 subjects while there were 352 subjects in the largest study. Of the 79 studies, 77 studies reported the effect of soy product consumption on total cholesterol, 67 studies on LDL cholesterol, 78 studies on HDL cholesterol and 72 studies on triglycerides.

The main source of soy protein administered in the studies was highly refined soy products: ISP was used in 63 studies, SPC was used in 2 studies and soy beverages made from ISP were used in 3 studies. Other soy foods were administered to a lesser extent: soy beverages made from whole soy beans were used in 2 studies, TSP was

used in 1 study, SF was used in 2 studies, tofu was used alone in 1 study and in combination with other soy foods in another study and soy nuts were used in 6 studies. Fifty-three studies reported the isoflavone content of the soy treatments and 8 studies reported using isoflavone-depleted soy protein (ethanol washed soy protein). Control foods consisted of casein, milk proteins, control diet without the soy food, or animal protein (meat).

In 60 studies, soy foods were used for partial or total replacement of animal protein in the usual diet or in the context of diets designed to reduce cholesterol (e.g., the U.S. National Institutes of Health – National Heart, Lung, and Blood Institute (NIH-NHLBI) National Cholesterol Education Program [60] and the NIH-NHLBI Therapeutic Lifestyle Changes guide [61]). In 19 studies, soy products were consumed as supplements to the usual diet. Supplements typically do not displace other foods but are consumed in addition to the regular diet; whether or not displacement occurred was not discussed or measured in these studies. Of the 79 studies, 41 studies reported balanced caloric and macronutrients profiles between the control and the treatment groups.

The quality of the studies was assessed by the Food Directorate using SIGN criteria [62] to identify sources of potential bias with respect to randomization, allocation concealment, blinding and attrition. Also, studies were rated as lower quality if caloric and macronutrients profiles of the diets were not balanced between the control and the treatment groups. Twenty-three of the 79 studies (29%) were rated as higher quality.

### 2.3 Assessment of Causality

For the assessment of the food-health effect relationship, the consistency in the direction of effect and the strength of the association are rated. The consistency in the direction of effect indicates whether or not the health outcome is changing in a favourable direction with exposure to the food across studies. The strength of the association rates the association between the food and health outcome by considering the proportion of studies that showed statistical significance at  $p < 0.05$  among all included studies. As per Health Canada's *Guidance Document for Preparing a Submission for Food Health Claims* [2], consistency and strength of association ratings are categorized as high ( $\geq 75\%$  of studies), moderate (60-74% of studies), or low ( $< 60\%$  of studies).

The direction of effect was highly consistent towards a reduction in total cholesterol (75% of studies) and LDL cholesterol (81% of studies) levels when soy foods were consumed. A low proportion of studies showed a statistically significant reduction in total cholesterol (26% of studies) and LDL cholesterol (33% of studies) levels. These

conclusions were similar when only higher quality studies were taken into account.

The direction of effect was moderately consistent towards an increase in HDL cholesterol levels but highly consistent when only higher quality studies were taken into account. Fifteen percent (15%) of studies showed a statistically significant increase in HDL cholesterol levels. The direction of effect was moderately consistent towards a reduction in triglyceride levels. Eleven percent (11%) of studies showed a statistically significant reduction in triglyceride levels. These conclusions were similar when only higher quality studies were taken into account.

Health Canada's health claim substantiation process provides for the use of a meta-analysis if judged appropriate and useful to demonstrate an association between the food or food constituent and the health effect [2]. A meta-analysis was provided by the applicant and it was reproduced and expanded by the Food Directorate to include studies not captured in the petitioner's systematic review and meta-analysis.

The Food Directorate's meta-analysis pooled studies investigating the effect of various foods and ingredients that are sources of soy protein because there is substantial scientific evidence suggesting that the soy proteins and associated isoflavones, are the major contributors to the cholesterol-lowering effect of soy foods [63]. In addition, there is no evidence that the cholesterol-lowering effect is attributable to only one category of soy foods. The Food Directorate's approach of using a meta-analysis is similar to that taken by the Agency for Healthcare Research and Quality (AHRQ) who assessed the effects of soy on several health outcomes, including cholesterol lowering [64], although not for the purpose of a health claim assessment.

The Cochrane Information Management System's Review Manager 5.2 Software [65] was used to estimate weighted mean differences, using the generic inverse variance method and random effect model. Mean differences were calculated using mean end-of-treatment values. When mean end-of-treatment values were not provided, they were calculated using mean baseline and change values. Some references identified as part of the systematic review were not included in the meta-analysis because they did not report numerical values for the outcomes of interest measured [29] or only provided graphs from which values could not be extracted with confidence [24, 26, 37, 44, 57]. One reference was also excluded only from the meta-analysis for LDL cholesterol levels because numerical values were not reported for LDL cholesterol levels [13] and another reference [20] was excluded only from the meta-analyses for total cholesterol and TG levels because graphs were the only results reported for these

outcomes. For references with multiple soy treatment arms but a single non-soy arm, it was assumed that the mean differences (and their standard deviations) are independent of each other despite their shared control groups. The data for these studies was entered into the software as if each treatment was a separate study. In this case, the mean value for the control group was used as the comparator for each study and the number of participants in the control group was divided by the number of soy treatment groups to ensure that the weight attributed to each mean value was appropriate [64, 66]. Heterogeneity between trial results was assessed using the  $I^2$  statistic [66].  $I^2$  values over 50% or between 25% and 50% indicate substantial or moderate heterogeneity, respectively. The symmetry of funnel plots was examined for evidence of potential bias due to selective reporting.

Subgroup analysis was undertaken to investigate the influence of the following factors on the cholesterol-lowering effect of soy protein-containing foods: baseline cholesterol levels (elevated total cholesterol levels > 5.2 mmol/L vs. normal total cholesterol levels < 5.2 mmol/L); source of soy protein (highly refined soy products (e.g., ISP, SPC, soy beverages made from ISP) vs. soy foods (e.g., soy beverages made from whole soy beans, tofu, soy flour, TSP, soy nuts)); isoflavones (present vs. depleted); study design (parallel vs. crossover); sex (male vs. female); type of diet (usual vs. recommended for heart health); pattern of consumption (as supplement vs. as replacement of animal protein in the diet); quality of studies (higher vs. lower) and balancing of caloric and macronutrients profiles between the control and the treatment groups (balanced vs. not balanced).

The meta-analysis included a total of 68, 59, 68 and 64 studies from 42, 38, 43 and 40 references for total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride levels, respectively.

The weighted mean difference was -0.15 mmol/L (95% CI, -0.21 to -0.08),  $p < 0.00001$ ) for total cholesterol levels and -0.15 mmol/L (95% CI, -0.19 to -0.11,  $p < 0.00001$ ) for LDL cholesterol levels, representing approximately 2.6% and 4% reductions, respectively. While there was a moderate to substantial heterogeneity ( $I^2=59%$ ) among trials that reported on total cholesterol values, no statistical heterogeneity was observed ( $I^2=0%$ ) between the trials reporting on LDL cholesterol levels.

For total cholesterol, the magnitude of effect was significantly higher in hypercholesterolemic subjects (-0.21 mmol/L (95% CI, -0.28 to -0.13,  $p < 0.00001$ )) compared to normocholesterolemic subjects (-0.06 mmol/L (95% CI, -0.13 to 0.01,  $p=0.08$ )). The cholesterol lowering effect was no longer statistically significant when studies using isoflavone-depleted ISP were analysed separately.

For LDL cholesterol, the results of subgroup analyses showed that baseline cholesterol levels, source of soy protein, study design, sex (male vs. female); type of diet, pattern of consumption, quality of studies and balancing of caloric and macronutrients profiles between the control and the treatments groups had little influence on the weighted mean difference and its statistical significance. In contrast, the pooled mean difference for LDL cholesterol was no longer statistically significant when only the studies using isoflavone-depleted ISP were considered.

The changes in HDL cholesterol levels (+0.03 mmol/L (95% CI, +0.01 to 0.06,  $p=0.002$ )) and in triglyceride levels (-0.06 mmol/L (95% CI, -0.10 to -0.02,  $p=0.001$ )) were statistically significant in the overall analysis.

The evidence consistently supports a direction of effect towards a reduction in total and LDL cholesterol levels when soy protein-containing foods are consumed as a replacement of animal protein and/or as a supplement to the usual diet. A meta-analysis showed a statistically significant reduction in total and LDL cholesterol levels with soy protein-containing food consumption and no detrimental effect on HDL cholesterol and triglyceride levels.

The estimated magnitude of total cholesterol and LDL cholesterol lowering in this meta-analysis is comparable to the magnitude of effect reported in other meta-analyses investigating the cholesterol-lowering effect of soy product consumption and using similar selection criteria with respect to study population, health status, soy food category and study quality [64, 67, 68]. Reductions ranging from 0.13 to 0.22 mmol/L for total cholesterol levels and from 0.09 to 0.23 mmol/L for LDL cholesterol levels have been reported.

Criteria for the establishment of a magnitude of cholesterol lowering that is considered biologically or clinically significant have not been identified or agreed upon by clinicians. Epidemiological and intervention data suggest that for every 1% reduction in LDL cholesterol there is a corresponding 1-2% reduction in cardiovascular events (CVEs), and for every 2-3% increase in HDL cholesterol there is a reduction in CVEs by 2-4% that is independent of LDL cholesterol [69-71]. Thus, even modest changes in cholesterol levels can be meaningful.

While there is consistent evidence for the cholesterol-lowering effect of soy protein, data on the contribution of soy isoflavones to cholesterol lowering is inconsistent [63]. Because of the difficulties in accurately isolating the effect of each constituent of soy protein-containing food products, soy protein is used as the main qualifier to assess the effect of the consumption of these foods on

cholesterol lowering. This is particularly important when communicating the health benefit to consumers with a claim indicating the amount of soy protein needed to lower cholesterol.

No apparent dose-response relationship was observed when LDL cholesterol reductions (the main target for cholesterol lowering strategies) were plotted against the daily intake of soy protein. Part of the reason for this may be that many studies used similar daily doses of soy protein (25-50 g/day). It is also possible that components other than soy proteins contribute to the hypocholesterolemic effect of soy products. In the absence of data to support a dose-response relationship, the dose most commonly used in the studies reviewed of 25 g of soy protein per day is chosen as the minimum effective daily intake.

### 3. Dietary Exposure and Feasibility of Consuming an Effective Amount of the Food

Soy protein consumption by Canadian adults was estimated based on the Canadian Community Health Survey (CCHS 2.2, 2004). Among 20,156 respondents, aged ≥19 years, 5.2% reported consuming soy products. Among eaters only (n=1048), the average quantity of soy protein consumed was 9.28 ± 0.81 g/day (95% CI, 7.68 to 10.88 g/day), based on a one day intake. About 34% of soy protein consumed was from soy flour.

Although the current soy protein intake among eaters is far from meeting the effective intake of 25 g of soy protein per day, several of the included studies show that it is feasible to consume 25 g/day of soy protein, for example, as a replacement for animal protein in the diet. Health Canada considers the consumption of 25 g of soy protein per day from soy protein-containing foods to be feasible for Canadians through 4 servings of foods containing a minimum of 6 g of soy protein per serving. This is equivalent to approximately 1.5 Food Guide servings of tofu per day (225 g) or 3-4 servings per day of a fortified soy beverage containing at least 6 g of soy protein per serving.

### 4. Proposed Claim Wording

To communicate the intended health effects of protein-containing soy products to the public, clear and accurate wording for the claim is proposed. This proposal is supported by the outcome of focus groups conducted with consumers and health professionals to help determine the wording of similar cholesterol-lowering claims for oat products, psyllium products and plant sterols. Health professionals (e.g., dietitians) and consumers favoured simple and direct statements over lengthier health claims.

The health claim recommended includes a primary statement as well as three additional statements.

The primary statement would be structured as follows<sup>2</sup>:

[*Serving size from Nutrition Facts table in metric and common household measures*] of (*brand name*) [*name of food*] supplies/provides X% of the daily amount of soy protein shown to help reduce/lower cholesterol.

For example<sup>3</sup>:

150 g of tofu supplies 70% of the daily amount of soy protein shown to help lower cholesterol.

250 mL (1 cup) of enriched soy beverage supplies 30% of the daily amount of soy protein shown to help lower cholesterol.

The “daily amount” referred to in the primary statement is 25 g of soy protein. This amount is based on the evidence available concerning the amount of soy protein shown to help reduce cholesterol. In this statement, the percentage of the daily amount of soy protein provided in one serving should be expressed to the nearest multiple of 5%.

The following additional statements could be placed adjacent to the primary statement, in letters up to twice the size and prominence of those in the primary statement:

- Soy protein helps reduce/lower cholesterol
- High cholesterol is a risk factor for heart disease
- Soy protein helps reduce/lower cholesterol, (which is) a risk factor for heart disease

### 5. Proposed Conditions of Use

Health Canada is proposing the following conditions for foods to carry the claim:

- a) The food contains at least 6 g of soy protein<sup>4</sup>
  - i. per reference amount and per serving of stated size, or
  - ii. per serving of stated size, if the food is a prepackaged meal.

<sup>2</sup> [ ] = mandatory; ( ) = optional; / = acceptable alternate wording.

<sup>3</sup> Examples are for illustration purposes only. They do not necessarily reflect acceptable health claims.

<sup>4</sup> As per B.01.305(1) of the *Food and Drug Regulations* (FDR), the food must also meet the requirement of “source of protein” set out in column 2 of item 8 of the table following B.01.513. Therefore, a Reasonable Daily Intake (RDI) of the food must have a protein rating of 20 or more. When no RDI is established in Schedule K for the food, the reference amount can be used to calculate its protein rating. For soy beverages, it is acceptable to use the RDI of milk to calculate the protein rating based on the consideration that soy beverages are used as milk substitutes.



- b) The food contains soy protein that has not been treated (alcohol washed) to remove soy isoflavones.
- c) The food contains at least 10% of the weighted recommended nutrient intake (WRNI) of a vitamin or a mineral nutrient
  - i. per reference amount and per serving of stated size, or
  - ii. per serving of stated size, if the food is a prepackaged meal.
- d) The food contains 100 mg or less of cholesterol per 100 g of food.
- e) The food contains 0.5% or less of alcohol.
- f) The food contains
  - i. 480 mg or less of sodium per reference amount and per serving of stated size, and per 50 g if the reference amount is 30 g or 30 mL or less, or
  - ii. 960 mg or less of sodium per serving of stated size, if the food is a prepackaged meal.
- g) The food meets the conditions for “free of saturated fatty acids” or “low in saturated fatty acids” (Items 18 and 19, respectively, in the table following section B.01.513 of the *Food and Drug Regulations*).
- h) The food is enriched with calcium and vitamin D if it is a soy beverage.

Condition a) is proposed on the basis that consumption of the daily amount of 25 g of soy protein shown to lower cholesterol could be achieved through 4 servings of the food (25 g of soy protein divided by 4 servings equals approximately 6 g of soy protein per serving). This is in alignment with a similar claim authorized in the U.S. (minimum of 6.25 g of soy protein per reference amount customarily consumed). However, as stipulated in B.01.305(1) of the *Food and Drug Regulations* (FDR), foods carrying the proposed claim about soy protein will also need to meet the "source of protein" conditions (item 8 of the table following B.01.513), requiring certain foods to contain more than 6 g of soy protein per serving.

Condition b) is proposed based on the meta-analysis showing that the effect of soy protein on LDL cholesterol levels was no longer statistically significant in the subgroup of studies using isoflavone-depleted ISP.

Conditions c), d), e), f) and g) are common to other permitted cholesterol-lowering health claims in Canada. They are recommended to ensure that the foods carrying the health claim will contain a relevant amount of one or more other nutrients in addition to the soy protein and will not contain significant amounts of ingredients such as cholesterol, alcohol, sodium or saturated fat that could negate the food's ability to contribute to a healthy diet aimed at reducing the risk of heart disease in Canada.

Condition h) is proposed on the basis that soy beverages

are consumed as substitutes for cow's milk which is an important source of calcium and vitamin D in the diets of Canadians. Therefore, the nutritional profile of soy beverages should be similar to cow's milk for these nutrients.

## 6. International Context

Health Canada's conclusion is not consistent with the conclusions of the European Food Safety Authority (EFSA) concerning a health claim about soy protein and a reduction of LDL cholesterol levels [72, 73]. EFSA concluded that a cause and effect relationship has not been established between the consumption of soy protein [72] and ISP [73] and the reduction of LDL-cholesterol concentrations. The divergent conclusions are mainly related to differences in the approaches used to assess the validity of the proposed health claims but Health Canada also evaluated evidence from human intervention studies not included in the EFSA reviews.

One of the differences relates to the characterization of the food, specifically to the presence, in addition to soy protein, of food constituents that could also exert an effect on cholesterol levels (e.g., fibre, polyunsaturated fatty acids and isoflavones). EFSA rejected studies conducted using soy foods or isoflavone-containing ISP because of the presence of these other food constituents and because they did not test the effect of soy protein *per se*. In contrast, Health Canada assessed the proposed claim for all soy products (except soy sauce and soy oil) using soy protein as the main qualifier. The potential cholesterol-lowering effects of highly refined soy products (ISP, SPC, soy beverages made from ISP) and other soy foods (soy beverages made from whole soy beans, tofu, soy flour, TSP and soy nuts) was assessed separately, as part of subgroup analyses. The effect was statistically significant in both subgroups. The presence of isoflavones was also deemed important for the claim because the subgroup of studies using isoflavone-depleted ISP did not show a statistically significant effect.

Another difference between the assessments by EFSA and Health Canada relates to the specific types of information used for the substantiation of a proposed claim. EFSA's decision is based on the fact that only a few studies showed a statistically significant cholesterol-lowering effect and that the differences in results between studies could not be explained by the dose used, sample size or study duration. In addition, EFSA stated that the evidence provided did not support the proposed mechanism by which the protein component of soy would exert the claimed effect [72, 73].

On the other hand, Health Canada's conclusion is based on a meta-analysis showing a statistically significant

cholesterol-lowering effect when soy products are consumed. While noting that no dose-response relationship was demonstrated between soy protein intake and the magnitude of cholesterol lowering, Health Canada also recognized that the range of doses used in many of the trials included in the meta-analysis was relatively narrow (25-50 g).

Lastly, while a plausible mechanism of action is desirable, convincing evidence of the mechanism of action is not essential to the substantiation of a health claim. Keeping in mind the broader inclusion of types of soy products in Health Canada's review, multiple mechanisms have been identified that may contribute to the cholesterol-lowering effects of soy proteins and their associated isoflavones, including (a) suppression of cholesterol biosynthesis, (b) increase of catabolism and excretion, and (c) reduction of uptake. Soy proteins or isoflavones reduced cholesterol synthesis via inhibition of the activity or gene expression of hydroxymethylglutaryl-CoA reductase, a rate-limiting enzyme in the cholesterol biosynthesis, in human HepG2 liver cells [74], MCF-7 breast cancer cells [75], and rats [76]. Feeding soy protein or soy peptides increased production and excretion of bile acids, derivatives of cholesterol, in rabbits [77], rats [78] and human volunteers [79], a process which may be regulated by increased gene expression or enzymatic activity of cholesterol 7-alpha-hydroxylase [63, 80]. Additionally, both soy protein and soy peptide up-regulated the expression of LDL receptor, which controls the uptake of circulating LDL cholesterol into the cells for catabolism, in human HepG2 liver cells [81], animals [82], and type 2 diabetic patients [83]. Thus, potential mechanisms of action proposed on the basis of in vitro and in vivo animal studies have been supported by studies in humans (e.g. [79] and [83]) that were not reviewed by EFSA.

Health Canada's conclusion is in alignment with the U.S. Food and Drug Administration's (FDA) decision in 1999 to permit soy products to carry a claim about a reduced risk of heart disease [84]. The claim permitted is "25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of [name of food] supplies \_\_ grams of soy protein". In contrast to Health Canada's proposed claim, the FDA claim refers to the reduction of heart disease risk, using total and LDL cholesterol levels as surrogate endpoints for coronary heart disease.

The FDA reported in 2007 that it is re-evaluating the evidence in support of the soy and coronary heart disease claim [85].

## 7. Health Risks and Risk Management Options

Soy protein-containing foods and ingredients are food commodities with a long history of safe use in the North

American food supply. They are not novel foods and do not require further safety evaluation. Adverse effects of soy consumption reported in the studies included in the systematic review were generally minor and gastrointestinal in nature.

Soy is considered one of the priority food allergens in Canada [86]. The presence of soy must be clearly indicated on the label whenever soy is used as an ingredient in a food, as specified in the *Food and Drug Regulations*. These requirements provide soy allergic consumers with the information they need to make informed choices regardless of the presence of a health claim on the label [87].

Soybeans contain soybean trypsin inhibitors (SBTI) which have been shown to have adverse physiological effects (inhibition of digestive enzymes, effect on nutritional quality and bioavailability of proteins and amino acids, pancreatic problems) in both animals and humans [88, 89]. Relatively high SBTI activity has been detected in many soy beverages, suggesting that proper heat processing is not always being followed [90]. Health Canada is in the process of developing guidance to industry to limit the content of active SBTI remaining in soy products.

## 8. Conclusions

Health Canada has concluded that the scientific evidence supports a cholesterol-lowering effect for foods containing soy protein with their associated soy isoflavones.

It is understood that the claimed effect in the proposed claim is cholesterol lowering in the context of a total diet. The claim is not intended as a clinical guideline for cholesterol and heart disease management, and the magnitude of effect would understandably be expected to be lower than for drugs. In the long term, the estimated reduction in LDL cholesterol levels associated with the consumption of soy products can have a significant impact in reducing cholesterol concentrations at the population level, an important modifiable risk factor for cardiovascular disease [91].

## 9. References

- [1] Health Canada. Health Claim Assessments. Available: <http://www.hc-sc.gc.ca/fn-an/label-etiquet/claims-reclam/assess-evalu/index-eng.php>. Accessed 2014 Jul 31.
- [2] Health Canada (2009) Guidance Document for Preparing a Submission for Food Health Claims. Available: [http://hc-sc.gc.ca/fn-an/legislation/guide-ld/health-claims\\_guidance-orientation\\_allegations-sante-eng.php](http://hc-sc.gc.ca/fn-an/legislation/guide-ld/health-claims_guidance-orientation_allegations-sante-eng.php). Accessed 2014 Jul 29.

- [3] Health Canada. Canadian Nutrient File (CNF) - Search by Food. Available: <http://webprod3.hc-sc.gc.ca/cnf-fce/start-debuter.do?lang=eng>. Accessed 2014 Jul 30.
- [4] Messina M (2010) Insights Gained from 20 Years of Soy Research. *J. Nutr.* 140: 2289S-2295S.
- [5] Messina M, Lane B (2007) Soy Protein, Soybean Isoflavones and Coronary Heart Disease Risk: Where do we Stand? *Future Lipidol.* 2: 55-74.
- [6] Public Health Agency of Canada. Minimizing the Risks of Cardiovascular Disease. Available: <http://www.phac-aspc.gc.ca/cd-mc/cvd-mcv/risk-riques-eng.php>. Accessed 2014 Jul 31.
- [7] Statistics Canada. The 10 Leading Causes of Death, 2011. Available: <http://www.statcan.gc.ca/pub/82-625-x/2014001/article/11896-eng.htm>. Accessed 2014 Jul 29.
- [8] Woolf KJ, Block RC (2012) Dyslipidemia. In: Bisognano JD, Beck GR, Connell RW, editors. *Manual of Outpatient Cardiology*. London: Springer London Dordrecht Heidelberg New York. pp. 159-178.
- [9] Anderson TJ, Grégoire J, Hegele RA, Couture P, Mancini GBJ, McPherson R, Francis GA, Poirier P, Lau DC, Grover S, Genest J, Jr, Carpentier AC, Dufour R, Gupta M, Ward R, Leiter LA, Lonn E, Ng DS, Pearson GJ, Yates GM, Stone JA, Ur E (2013) 2012 Update of Canadian Cardiovascular Society Guidelines for the Diagnosis and Treatment of Dyslipidemia for the Prevention of Cardiovascular Disease in the Adult. *Can. J. Cardiol.* 29: 151-167.
- [10] Statistics Canada. Cholesterol Levels of Canadians, 2009 to 2011. Available: <http://www.statcan.gc.ca/pub/82-625-x/2012001/article/11732-eng.htm>. Accessed 2014 Aug 29.
- [11] Bakhtiary A, Yassin Z, Hanachi P, Rahmat A, Ahmad Z, Jalali F (2010) Effects of Soy on Metabolic Biomarkers of Cardiovascular Disease in Elderly Women with the Metabolic Syndrome. *J. Family Reprod. Health.* 4: 95-104.
- [12] Allen JK, Becker DM, Kwiterovich PO, Lindenstruth KA, Curtis C (2007) Effect of Soy Protein-Containing Isoflavones on Lipoproteins in Postmenopausal Women. *Menopause.* 14: 106-114.
- [13] Ashton EL, Dalais FS, Ball MJ (2000) Effect of Meat Replacement by Tofu on CHD Risk Factors Including Copper Induced LDL Oxidation. *J. Am. Coll. Nutr.* 19: 761-767.
- [14] Azadbakht L, Kimiagar M, Mehrabi Y, Esmailzadeh A, Padyab M, Hu FB, Willett WC (2007) Soy Inclusion in the Diet Improves Features of the Metabolic Syndrome: A Randomized Crossover Study in Postmenopausal Women. *Am. J. Clin. Nutr.* 85: 735-741.
- [15] Bakhit RM, Klein BP, Essex-Sorlie D, Ham JO, Erdman JW, Jr, Potter SM (1994) Intake of 25 g of Soybean Protein with or without Soybean Fiber Alters Plasma Lipids in Men with Elevated Cholesterol Concentrations. *J. Nutr.* 124: 213-222.
- [16] Basaria S, Wisniewski A, Dupree K, Bruno T, Song MY, Yao F, Ojumu A, John M, Dobs AS (2009) Effect of High-Dose Isoflavones on Cognition, Quality of Life, Androgens, and Lipoprotein in Post-Menopausal Women. *J. Endocrinol. Invest.* 32: 150-155.
- [17] Baum JA, Teng H, Erdman JW, Jr, Weigel RM, Klein BP, Persky VW, Freels S, Surya P, Bakhit RM, Ramos E, Shay NF, Potter SM (1998) Long-Term Intake of Soy Protein Improves Blood Lipid Profiles and Increases Mononuclear Cell Low-Density-Lipoprotein Receptor Messenger RNA in Hypercholesterolemic, Postmenopausal Women. *Am. J. Clin. Nutr.* 68: 545-551.
- [18] Blum A, Lang N, Vigder F, Israeli P, Gumanovsky M, Lupovitz S, Elgazi A, Peleg A, Ben-Ami M (2003) Effects of Soy Protein on Endothelium-Dependent Vasodilatation and Lipid Profile in Postmenopausal Women with Mild Hypercholesterolemia. *Clin. Invest. Med.* 26: 20-26.
- [19] Borodin EA, Menshikova IG, Dorovskikh VA, Feoktistova NA, Shtarberg MA, Yamamoto T, Takamatsu K, Mori H, Yamamoto S (2009) Effects of Two-Month Consumption of 30 g a Day of Soy Protein Isolate or Skimmed Curd Protein on Blood Lipid Concentration in Russian Adults with Hyperlipidemia. *J. Nutr. Sci. Vitaminol. (Tokyo).* 55: 492-497.
- [20] Bricarello LP, Kasinski N, Bertolami MC, Faludi A, Pinto LA, Relvas WG, Izar MC, Ihara SS, Tufik S, Fonseca FA (2004) Comparison between the Effects of Soy Milk and Non-Fat Cow Milk on Lipid Profile and Lipid Peroxidation in Patients with Primary Hypercholesterolemia. *Nutrition.* 20: 200-204.
- [21] Campbell SC, Khalil DA, Payton ME, Arjmandi BH (2010) One-Year Soy Protein Supplementation does Not Improve Lipid Profile in Postmenopausal Women. *Menopause.* 17: 587-593.
- [22] Crouse JR, 3rd, Morgan T, Terry JG, Ellis J, Vitolsins M, Burke GL (1999) A Randomized Trial Comparing the Effect of Casein with that of Soy Protein Containing Varying Amounts of Isoflavones on Plasma Concentrations of Lipids and Lipoproteins. *Arch. Intern. Med.* 159: 2070-2076.
- [23] Cuevas AM, Iribarra VL, Castillo OA, Yanez MD, Germain AM (2003) Isolated Soy Protein Improves Endothelial Function in Postmenopausal Hypercholesterolemic Women. *Eur. J. Clin. Nutr.* 57: 889-894.
- [24] Dent SB, Peterson CT, Brace LD, Swain JH, Reddy MB, Hanson KB, Robinson JG, Alekel DL (2001) Soy Protein Intake by Perimenopausal Women does not Affect Circulating Lipids and Lipoproteins or Coagulation and Fibrinolytic Factors. *J. Nutr.* 131: 2280-2287.



- [25] Evans M, Njike VY, Hoxley M, Pearson M, Katz DL (2007) Effect of Soy Isoflavone Protein and Soy Lecithin on Endothelial Function in Healthy Postmenopausal Women. *Menopause*. 14: 141-149.
- [26] Gardner CD, Messina M, Kiazand A, Morris JL, Franke AA (2007) Effect of Two Types of Soy Milk and Dairy Milk on Plasma Lipids in Hypercholesterolemic Adults: A Randomized Trial. *J. Am. Coll. Nutr.* 26: 669-677.
- [27] Gardner CD, Newell KA, Cherin R, Haskell WL (2001) The Effect of Soy Protein with or without Isoflavones Relative to Milk Protein on Plasma Lipids in Hypercholesterolemic Postmenopausal Women. *Am. J. Clin. Nutr.* 73: 728-735.
- [28] Giovannetti PM, Carroll KK, Wolfe BM (1986) Constancy of Fasting Serum Cholesterol of Healthy Young Women upon Substitution of Soy Protein Isolate for Meat and Dairy Protein in Medium and Low Fat Diets. *Nutr. Res.* 6: 609-618.
- [29] Gooderham MH, Adlercreutz H, Ojala ST, Wahala K, Holub BJ (1996) A Soy Protein Isolate Rich in Genistein and Daidzein and its Effects on Plasma Isoflavone Concentrations, Platelet Aggregation, Blood Lipids and Fatty Acid Composition of Plasma Phospholipid in Normal Men. *J. Nutr.* 126: 2000-2006.
- [30] Higashi K, Abata S, Iwamoto N, Ogura M, Yamashita T, Ishikawa O, Ohlszu F, Nakamura H (2001) Effects of Soy Protein on Levels of Remnant-Like Particles Cholesterol and Vitamin E in Healthy Men. *J. Nutr. Sci. Vitaminol. (Tokyo)*. 47: 283-288.
- [31] Hoie LH, Graubaum HJ, Harde A, Gruenwald J, Wernecke KD (2005) Lipid-Lowering Effect of 2 Dosages of a Soy Protein Supplement in Hypercholesterolemia. *Adv. Ther.* 22: 175-186.
- [32] Hoie LH, Morgenstern EC, Gruenwald J, Graubaum HJ, Busch R, Luder W, Zunft HJ (2005) A Double-Blind Placebo-Controlled Clinical Trial Compares the Cholesterol-Lowering Effects of Two Different Soy Protein Preparations in Hypercholesterolemic Subjects. *Eur. J. Nutr.* 44: 65-71.
- [33] Jassi HK, Jain A, Arora S, Chitra R (2010) Effect of Soy Proteins vs Soy Isoflavones on Lipid Profile in Postmenopausal Women. *Indian J. Clin. Biochem.* 25: 201-207.
- [34] Kurowska EM, Jordan J, Spence JD, Wetmore S, Piche LA, Radzikowski M, Dandona P, Carroll KK (1997) Effects of Substituting Dietary Soybean Protein and Oil for Milk Protein and Fat in Subjects with Hypercholesterolemia. *Clin. Invest. Med.* 20: 162-170.
- [35] Lichtenstein AH, Jalbert SM, Adlercreutz H, Goldin BR, Rasmussen H, Schaefer EJ, Ausman LM (2002) Lipoprotein Response to Diets High in Soy or Animal Protein with and without Isoflavones in Moderately Hypercholesterolemic Subjects. *Arterioscler. Thromb. Vasc. Biol.* 22: 1852-1858.
- [36] Ma L, Grann K, Li M, Jiang Z (2011) A Pilot Study to Evaluate the Effect of Soy Isolate Protein on the Serum Lipid Profile and Other Potential Cardiovascular Risk Markers in Moderately Hypercholesterolemic Chinese Adults. *Ecol. Food Nutr.* 50: 473-485.
- [37] Ma Y, Chiriboga D, Olendzki BC, Nicolosi R, Merriam PA, Ockene IS (2005) Effect of Soy Protein Containing Isoflavones on Blood Lipids in Moderately Hypercholesterolemic Adults: A Randomized Controlled Trial. *J. Am. Coll. Nutr.* 24: 275-285.
- [38] Matthan NR, Jalbert SM, Ausman LM, Kuvin JT, Karas RH, Lichtenstein AH (2007) Effect of Soy Protein from Differently Processed Products on Cardiovascular Disease Risk Factors and Vascular Endothelial Function in Hypercholesterolemic Subjects. *Am. J. Clin. Nutr.* 85: 960-966.
- [39] McVeigh BL, Dillingham BL, Lampe JW, Duncan AM (2006) Effect of Soy Protein Varying in Isoflavone Content on Serum Lipids in Healthy Young Men. *Am. J. Clin. Nutr.* 83: 244-251.
- [40] Meinertz H, Nilausen K, Faergeman O (1990) Effects of Dietary Proteins on Plasma Lipoprotein Levels in Normal Subjects: Interaction with Dietary Cholesterol. *J. Nutr. Sci. Vitaminol. (Tokyo)*. 36: S157-64.
- [41] Meinertz H, Nilausen K, Hilden J (2002) Alcohol-Extracted, but Not Intact, Dietary Soy Protein Lowers Lipoprotein(a) Markedly. *Arterioscler. Thromb. Vasc. Biol.* 22: 312-316.
- [42] Nilausen K, Meinertz H (1998) Variable Lipemic Response to Dietary Soy Protein in Healthy, Normolipemic Men. *Am. J. Clin. Nutr.* 68: 1380S-1384S.
- [43] Potter SM, Bakhit RM, Essex-Sorlie DL, Weingartner KE, Chapman KM, Nelson RA, Prabhudesai M, Savage WD, Nelson AI, Winter LW (1993) Depression of Plasma Cholesterol in Men by Consumption of Baked Products Containing Soy Protein. *Am. J. Clin. Nutr.* 58: 501-506.
- [44] Potter SM, Baum JA, Teng H, Stillman RJ, Shay NF, Erdman JW, Jr (1998) Soy Protein and Isoflavones: Their Effects on Blood Lipids and Bone Density in Postmenopausal Women. *Am. J. Clin. Nutr.* 68: 1375S-1379S.
- [45] Santo AS, Cunningham AM, Alhassan S, Browne RW, Burton H, Leddy JJ, Grandjean PW, Horvath SM, Horvath PJ (2008) NMR Analysis of Lipoprotein Particle Size does not Increase Sensitivity to the Effect of Soy Protein on CVD Risk when Compared with the Traditional Lipid Profile. *Appl. Physiol. Nutr. Metab.* 33: 489-500.
- [46] Shidfar F, Eshramphosh E, Heydari I, Haghghi L, Hosseini S, Shidfar S (2009) Effects of Soy Bean on Serum Paraoxonase 1 Activity and Lipoproteins in

- Hyperlipidemic Postmenopausal Women. *Int. J. Food Sci. Nutr.* 60: 195-205.
- [47] Shige H, Ishikawa T, Higashi K, Yamashita T, Tomiyasu K, Yoshida H, Hosoi H, Ito T, Nakajima K, Ayaori M, Yonemura A, Suzukawa M, Nakamura H (1998) Effects of Soy Protein Isolate (SPI) and Casein on the Postprandial Lipemia in Normolipidemic Men. *J. Nutr. Sci. Vitaminol. (Tokyo)*. 44: 113-127.
- [48] Shorey RL, Bazan B, Lo GS, Steinke FH (1981) Determinants of Hypocholesterolemic Response to Soy and Animal Protein-Based Diets. *Am. J. Clin. Nutr.* 34: 1769-1778.
- [49] Steinberg FM, Guthrie NL, Villablanca AC, Kumar K, Murray MJ (2003) Soy Protein with Isoflavones has Favorable Effects on Endothelial Function that are Independent of Lipid and Antioxidant Effects in Healthy Postmenopausal Women. *Am. J. Clin. Nutr.* 78: 123-130.
- [50] Teede HJ, Dalais FS, Kotsopoulos D, Liang YL, Davis S, McGrath BP (2001) Dietary Soy has both Beneficial and Potentially Adverse Cardiovascular Effects: A Placebo-Controlled Study in Men and Postmenopausal Women. *J. Clin. Endocrinol. Metab.* 86: 3053-3060.
- [51] Teixeira SR, Potter SM, Weigel R, Hannum S, Erdman JW, Jr, Hasler CM (2000) Effects of Feeding 4 Levels of Soy Protein for 3 and 6 Wk on Blood Lipids and Apolipoproteins in Moderately Hypercholesterolemic Men. *Am. J. Clin. Nutr.* 71: 1077-1084.
- [52] Thorp AA, Howe PR, Mori TA, Coates AM, Buckley JD, Hodgson J, Mansour J, Meyer BJ (2008) Soy Food Consumption does not Lower LDL Cholesterol in either Equol or Nonequol Producers. *Am. J. Clin. Nutr.* 88: 298-304.
- [53] van Raaij JM, Katan MB, Hautvast JG, Hermus RJ (1981) Effects of Casein Versus Soy Protein Diets on Serum Cholesterol and Lipoproteins in Young Healthy Volunteers. *Am. J. Clin. Nutr.* 34: 1261-1271.
- [54] van Raaij JM, Katan MB, West CE, Hautvast JG (1982) Influence of Diets Containing Casein, Soy Isolate, and Soy Concentrate on Serum Cholesterol and Lipoproteins in Middle-Aged Volunteers. *Am. J. Clin. Nutr.* 35: 925-934.
- [55] Vigna GB, Pansini F, Bonaccorsi G, Albertazzi P, Donega P, Zanotti L, De Aloysio D, Mollica G, Fellin R (2000) Plasma Lipoproteins in Soy-Treated Postmenopausal Women: A Double-Blind, Placebo-Controlled Trial. *Nutr. Metab. Cardiovasc. Dis.* 10: 315-322.
- [56] Welty FK, Lee KS, Lew NS, Zhou JR (2007) Effect of Soy Nuts on Blood Pressure and Lipid Levels in Hypertensive, Prehypertensive, and Normotensive Postmenopausal Women. *Arch. Intern. Med.* 167: 1060-1067.
- [57] West SG, Hilpert KF, Juturu V, Bordi PL, Lampe JW, Mousa SA, Kris-Etherton PM (2005) Effects of Including Soy Protein in a Blood Cholesterol-Lowering Diet on Markers of Cardiac Risk in Men and in Postmenopausal Women with and without Hormone Replacement Therapy. *J. Womens Health. (Larchmt)*. 14: 253-262.
- [58] Wofford MR, Rebholz CM, Reynolds K, Chen J, Chen CS, Myers L, Xu J, Jones DW, Whelton PK, He J (2012) Effect of Soy and Milk Protein Supplementation on Serum Lipid Levels: A Randomized Controlled Trial. *Eur. J. Clin. Nutr.* 66: 419-425.
- [59] Wong WW, Smith EO, Stuff JE, Hachey DL, Heird WC, Pownell HJ (1998) Cholesterol-Lowering Effect of Soy Protein in Normocholesterolemic and Hypercholesterolemic Men. *Am. J. Clin. Nutr.* 68: 1385S-1389S.
- [60] National Institutes of Health - National Heart, Lung, and Blood Institute (2004) Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Available: <http://www.nhlbi.nih.gov/health-pro/guidelines/current/cholesterol-guidelines/index.htm>. Accessed 2014 Aug 22.
- [61] National Institutes of Health - National Heart, Lung, and Blood Institute (2005) Your Guide to Lowering Your Cholesterol with Therapeutic Lifestyle Changes. Available: [http://www.nhlbi.nih.gov/files/docs/public/heart/cho\\_l\\_tlc.pdf](http://www.nhlbi.nih.gov/files/docs/public/heart/cho_l_tlc.pdf). Accessed 2014 Aug 22.
- [62] Scottish Intercollegiate Guidelines Network (2011) SIGN 50: A Guideline Developer's Handbook. Available: <http://www.sign.ac.uk/guidelines/fulltext/50/index.html>. Accessed 2013 Sep 26.
- [63] Xiao CW, Mei J, Wood CM (2008) Effect of Soy Proteins and Isoflavones on Lipid Metabolism and Involved Gene Expression. *Front. Biosci.* 13: 2660-2673.
- [64] Balk E, Chung M, Chew P, Ip S, Raman G, Kupelnick B, Tatsioni A, Sun Y, Wolk B, DeVine D, Lau J (2005) Effects of Soy on Health Outcomes. *Evid. Rep. Technol. Assess.* 126: 1-8.
- [65] Cochrane Informatics & Knowledge Management Department. Review Manager. Available: <http://tech.cochrane.org/Revman>. Accessed 2014 Aug 22.
- [66] Higgins JPT, Green S (2011) Cochrane Handbook for Systematic Reviews of Interventions. Version 5.1.0. The Cochrane Collaboration. Available: [www.cochrane-handbook.org](http://www.cochrane-handbook.org).
- [67] Harland JL, Haffner TA (2008) Systematic Review, Meta-Analysis and Regression of Randomised Controlled Trials Reporting an Association between an Intake of Circa 25 g Soya Protein Per Day and Blood Cholesterol. *Atherosclerosis*. 200: 13-27.

- [68] Weggemans RM, Trautwein EA (2003) Relation between Soy-Associated Isoflavones and LDL and HDL Cholesterol Concentrations in Humans: A Meta-Analysis. *Eur. J. Clin. Nutr.* 57: 940-946.
- [69] Pedersen TR, Olsson AG, Faergeman O, Kjekshus J, Wedel H, Berg K, Wilhelmsen L, Haghfelt T, Thorgeirsson G, Pyorala K, Miettinen T, Christophersen B, Tobert JA, Musliner TA, Cook TJ (1998) Lipoprotein Changes and Reduction in the Incidence of Major Coronary Heart Disease Events in the Scandinavian Simvastatin Survival Study (4S). *Circulation.* 97: 1453-1460. [70] Gotto AM and Gundy SM (1999) Lowering LDL Cholesterol: Question from Recent Meta-Analyses of Clinical Trial Data Issues from Interdisciplinary Council on Reducing the Risk of Coronary Heart Disease. *Circulation.* 99 (8): E1-E7.
- [71] Charland SL, Malone DC (2010) Prediction of Cardiovascular Event Risk Reduction from Lipid Changes Associated with High Potency Dyslipidemia Therapy. *Curr. Med. Res. Opin.* 26: 365-375.
- [72] EFSA Panel on Dietetic Products, Nutrition and Allergies (2010) Scientific Opinion on the Substantiation of a Health Claim Related to Soy Protein and Reduction of Blood Cholesterol Concentrations Pursuant to Article 14 of the Regulation (EC) no 1924/2006. *EFSA Journal.* 8: 1688. Available: <http://www.efsa.europa.eu/en/efsajournal/doc/1688.pdf>.
- [73] EFSA Panel on Dietetic Products, Nutrition and Allergies (2012) Scientific Opinion on the Substantiation of a Health Claim Related to Isolated Soy Protein and Reduction of Blood LDL-Cholesterol Concentrations Pursuant to Article 14 of Regulation (EC) no 1924/2006. *EFSA Journal.* 10: 2555. Available: <http://www.efsa.europa.eu/en/efsajournal/doc/2555.pdf>.
- [74] Borradaile NM, De Dreu LE, Wilcox LJ, Edwards JY, Huff MW (2002) Soya Phytoestrogens, Genistein and Daidzein, Decrease Apolipoprotein B Secretion from HepG2 Cells through Multiple Mechanisms. *Biochem. J.* 366: 531-539.
- [75] Dyck JR, Kudo N, Barr AJ, Davies SP, Hardie DG, Lopaschuk GD (1999) Phosphorylation Control of Cardiac Acetyl-CoA Carboxylase by cAMP-Dependent Protein Kinase and 5'-AMP Activated Protein Kinase. *Eur. J. Biochem.* 262: 184-190.
- [76] Tovar AR, Murguía F, Cruz C, Hernandez-Pando R, Aguilar-Salinas CA, Pedraza-Chaverri J, Correa-Rotter R, Torres N (2002) A Soy Protein Diet Alters Hepatic Lipid Metabolism Gene Expression and Reduces Serum Lipids and Renal Fibrogenic Cytokines in Rats with Chronic Nephrotic Syndrome. *J. Nutr.* 132: 2562-2569.
- [77] Huff MW, Carroll KK (1980) Effects of Dietary Protein on Turnover, Oxidation, and Absorption of Cholesterol, and on Steroid Excretion in Rabbits. *J. Lipid Res.* 21: 546-548.
- [78] Sugano M, Goto S, Yamada Y, Yoshida K, Hashimoto Y, Matsuo T, Kimoto M (1990) Cholesterol-Lowering Activity of various Undigested Fractions of Soybean Protein in Rats. *J. Nutr.* 120: 977-985.
- [79] Wang MF, Yamamoto S, Chung HM, Chung SY, Miyatani S, Mori M, Okita T, Sugano M (1995) Antihypercholesterolemic Effect of Undigested Fraction of Soybean Protein in Young Female Volunteers. *J. Nutr. Sci. Vitaminol. (Tokyo).* 41: 187-195.
- [80] Gudbrandsen OA, Wergedahl H, Liaset B, Espe M, Berge RK (2005) Dietary Proteins with High Isoflavone Content Or Low Methionine-Glycine and Lysine-Arginine Ratios are Hypocholesterolaemic and Lower the Plasma Homocysteine Level in Male Zucker fa/fa Rats. *Br. J. Nutr.* 94: 321-330.
- [81] Lovati MR, Manzoni C, Gianazza E, Arnoldi A, Kurowska E, Carroll KK, Sirtori CR (2000) Soy Protein Peptides Regulate Cholesterol Homeostasis in Hep G2 Cells. *J. Nutr.* 130: 2543-2549.
- [82] Lovati MR, Manzoni C, Agostinelli P, Ciappellano S, Mannucci L, Sirtori CR (1991) Studies on the Mechanism of the Cholesterol Lowering Activity of Soy Proteins. *Nutr. Metab. Cardiovasc. Dis.* 1: 18-24.
- [83] Lovati MR, Manzoni C, Canavesi A, Sirtori M, Vaccarino V, Marchi M, Gaddi G, Sirtori CR (1987) Soybean Protein Diet Increases Low Density Lipoprotein Receptor Activity in Mononuclear Cells from Hypercholesterolemic Patients. *J. Clin. Invest.* 80: 1498-1502.
- [84] Food and Drug Administration (1999) Food Labeling: Health Claims; Soy Protein and Coronary Heart Disease. Final Rule. *Fed. Regist.* 64: 57700-57733.
- [85] Food and Drug Administration (2007) Health Claims and Qualified Health Claims; Dietary Lipids and Cancer, Soy Protein and Coronary Heart Disease, Antioxidant Vitamins and Certain Cancers, and Selenium and Certain Cancers; Reevaluation; Opportunity for Public Comment. *Fed. Regist.* 72: 72738-72740.
- [86] Health Canada (2013) Information for Canadians with Soy Allergy. Available: <http://www.hc-sc.gc.ca/fn-an/securit/allerg/fa-aa/soy-soya-eng.php>. Accessed 2014 Jul 30.
- [87] Health Canada. Food Allergen Labelling. Available: <http://www.hc-sc.gc.ca/fn-an/label-etiquet/allergen/index-eng.php>. Accessed 2014 Jul 30.
- [88] Food and Agriculture Organization of the United Nations (2013) Dietary Protein Quality Evaluation in Human Nutrition - Report of an FAO Expert Consultation. *FAO Food Nutr. Pap.* 92.
- [89] Gilani GS, Xiao CW, Cockell KA (2012) Impact of Antinutritional Factors in Food Proteins on the Digestibility of Protein and the Bioavailability of Amino Acids and on Protein Quality. *Br. J. Nutr.* 108: S315-S332.

- [90] Xiao CW, Wood C, Robertson P, Gilani GS (2012) Protease Inhibitor Activities and Isoflavone Content in Commercial Soymilks and Soy-Based Infant Formulas Sold in Ottawa, Canada. *J. Food Comp. Anal.* 25: 130-136.
- [91] Harland JI (2012) Food Combinations for Cholesterol Lowering. *Nutr. Res. Rev.* 25: 249-266.